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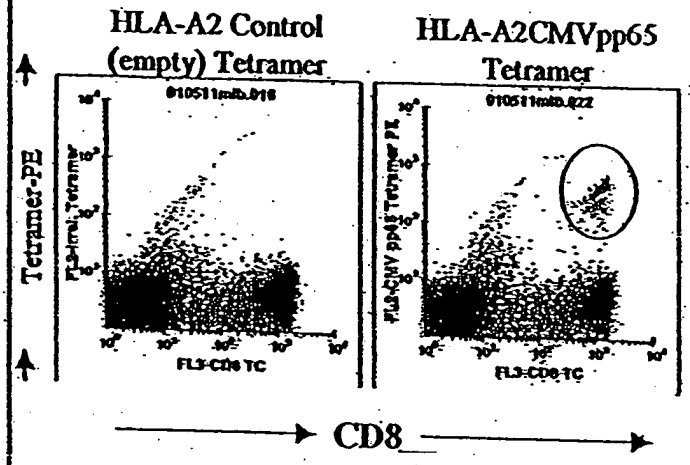
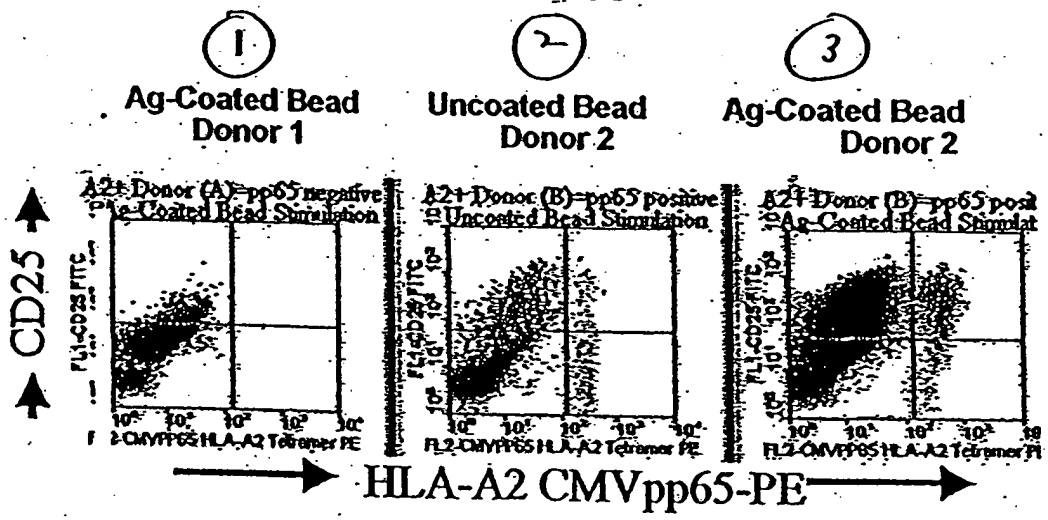
Flow Cytometric Analysis of HLA-A2+ Donor T Cells for HLA-A2 CMVpp65+ T Cells: Day 0 of Culture



Human PBMC were screened for HLA-A2 positivity. HLA-A2+ donors were screened with control (empty) HLA-A2 tetramers and CMVpp65 loaded tetramers. In the donor shown above, approximately 3% of the CD3+CD8+ express TCR specific for HLA-A2 CMVpp65.

FIG 1

Flow Cytometric Analysis of CD25 Expressio on HLA-A2 CMVpp65+ T Cells: Day 10 of Culture



(Tetramer Staining Gated on CD8+ T Cells)

PBMC were activated with CMV antigen (coated onto paramagnetic beads) and by day 10 of culture, many cell are shown to be CD25 (IL-2R) positive, and all of the HLA-A2 CMVpp65+ T cells are expressing high levels of CD25, indicating activation (right panel). Controls include the same donor cells treated with uncoated (antigen-negative) beads (middle panel), or an HLA-A2+ donor (donor 1) that did not show detectable tetramer+ cells at day 0 and was serologically negative for CMV (left panel). These data indicate that tetramer approaches can be effectively used to track antigen-specific T cells and their relative state of activation.

FIG. 2

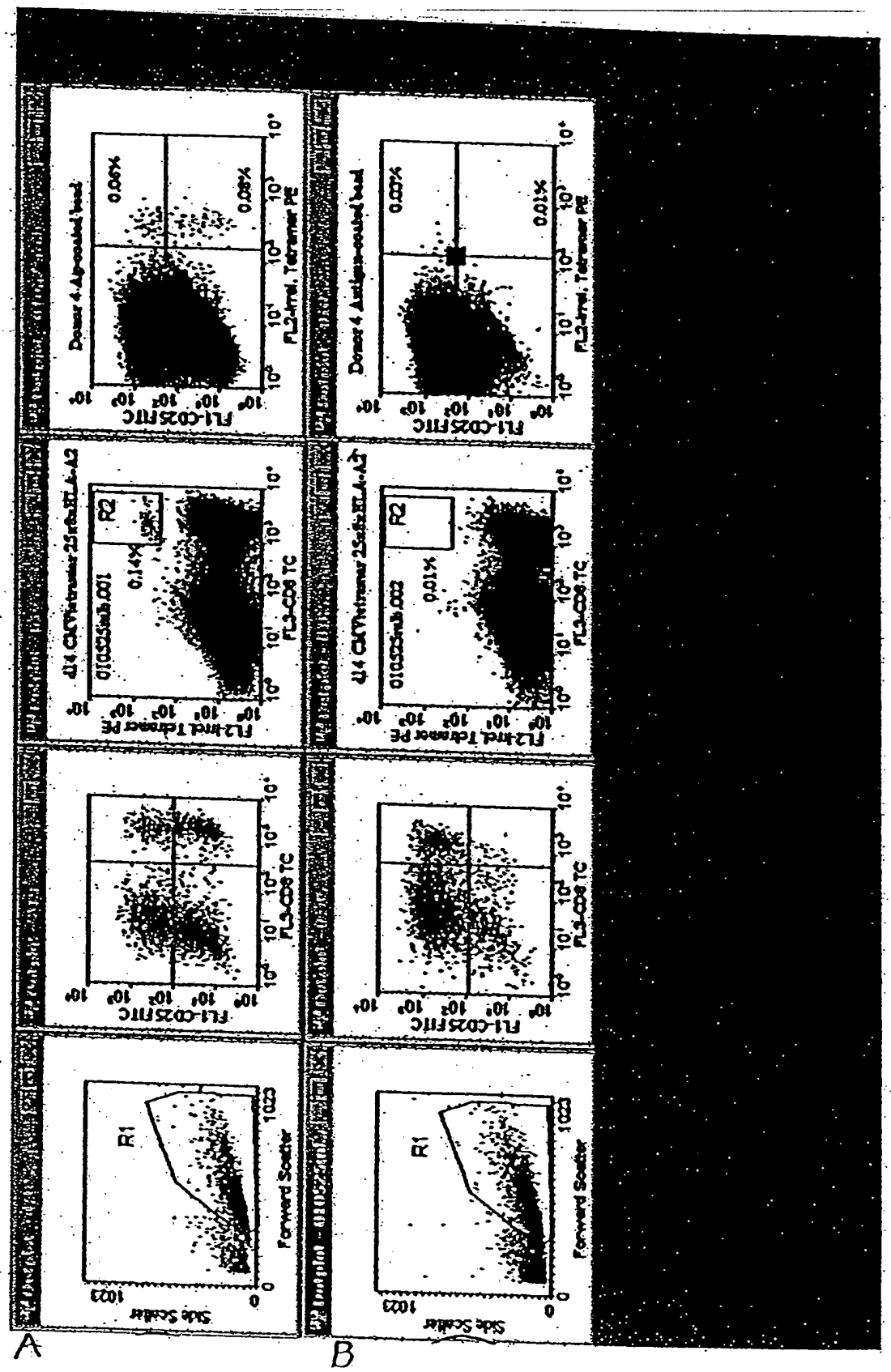

A
B
R1
R2
Forward Scatter
Side Scatter
FL1-CD25 FITC
FL2-Intl. Tetramer PE
FL3-CD8 TC

FIG 3

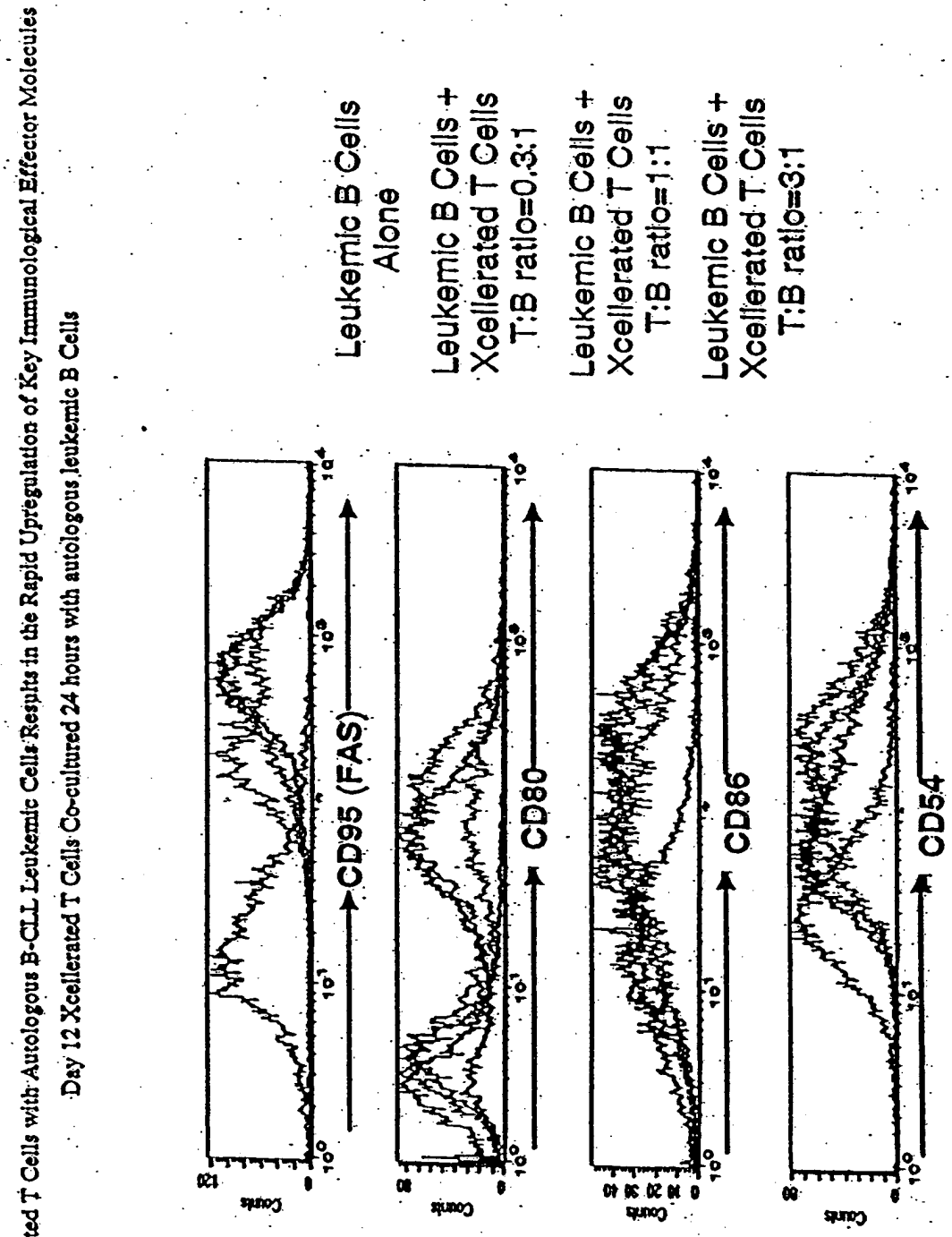

Mixing Xcellerated T Cells with Autologous B-CLL Leukemic Cells Results in the Rapid Upregulation of Key Immunological Effector Molecules
Day 12 Xcellerated T Cells Co-cultured 24 hours with autologous leukemic B Cells
Leukemic B Cells Alone
Leukemic B Cells + Xcellerated T Cells T:B ratio=0.3:1
Leukemic B Cells + Xcellerated T Cells T:B ratio=1:1
Leukemic B Cells + Xcellerated T Cells T:B ratio=3:1
CD95 (FAS)
CD80
CD86
CD54
Counts

FIG 4

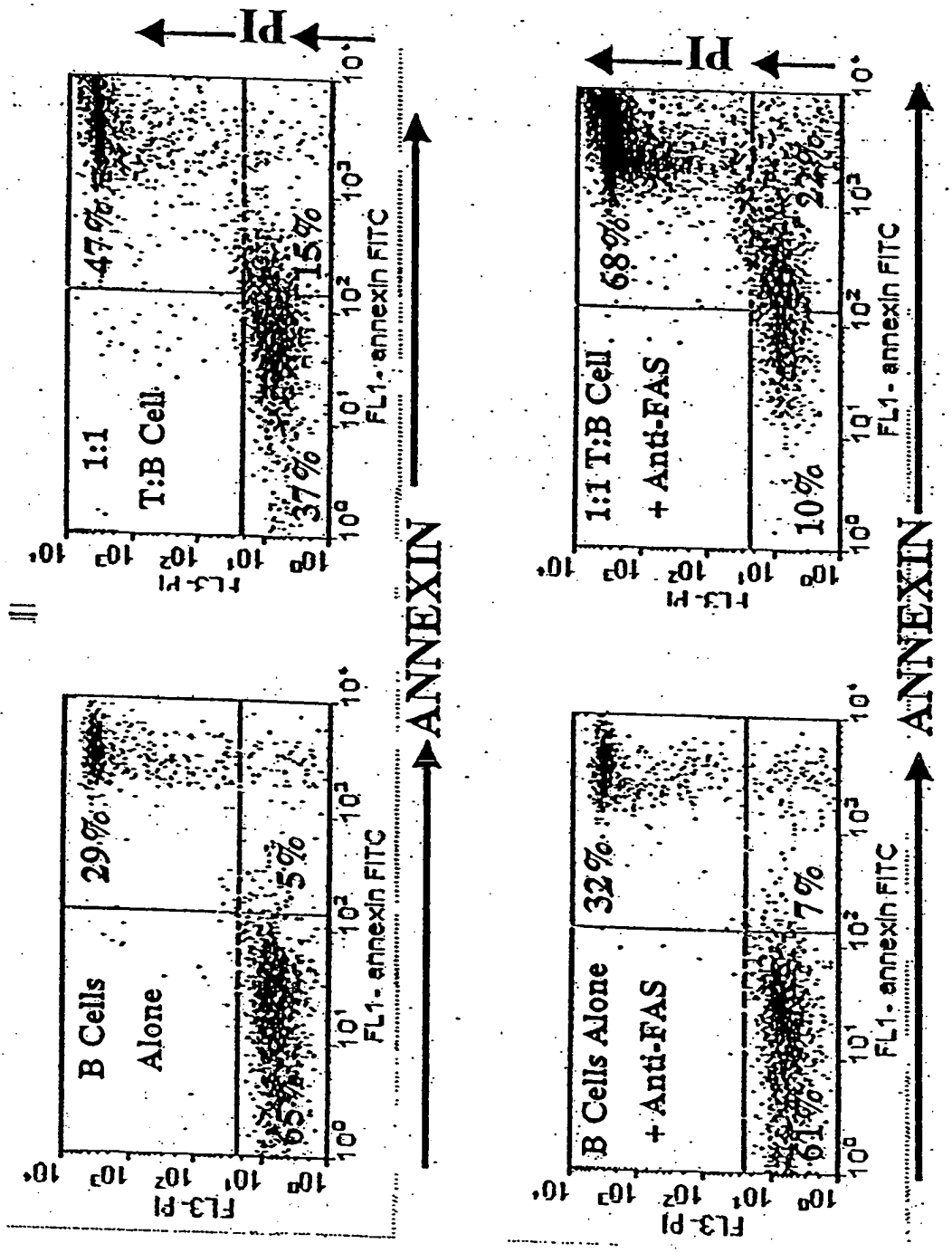

Id
1:1 T:B Cell
47%
15%
37%
FL1- annexin FITC
FL3- PI
ANNEXIN
1:1 T:B Cell
+ Anti-FAS
68%
22%
10%
B Cells Alone
29%
5%
65%
B Cells Alone
+ Anti-FAS
32%
7%
61%

FIG 5

T Cells Grow and Tumor Cells Are Eliminated During the Xcellerate Process

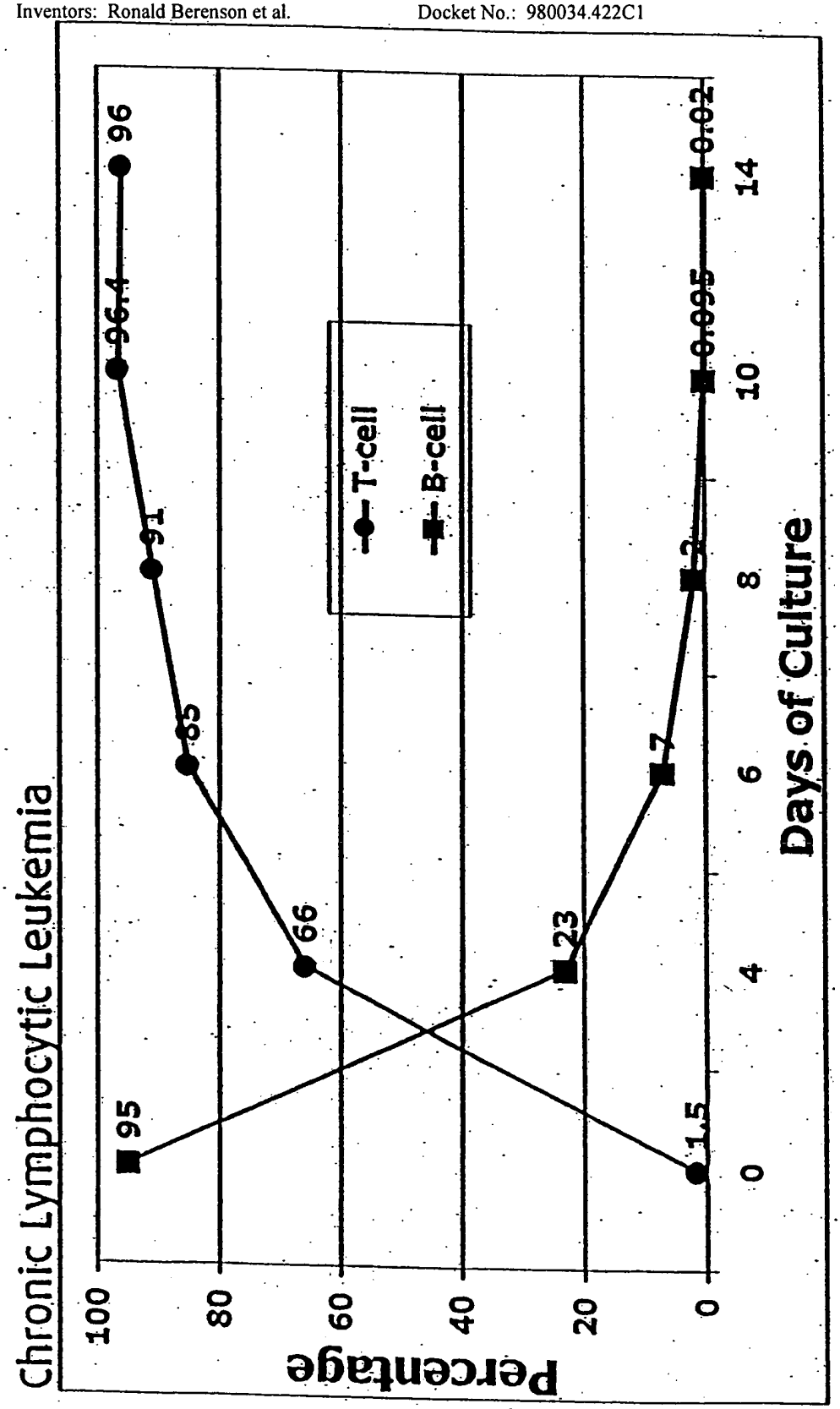


FIG 6

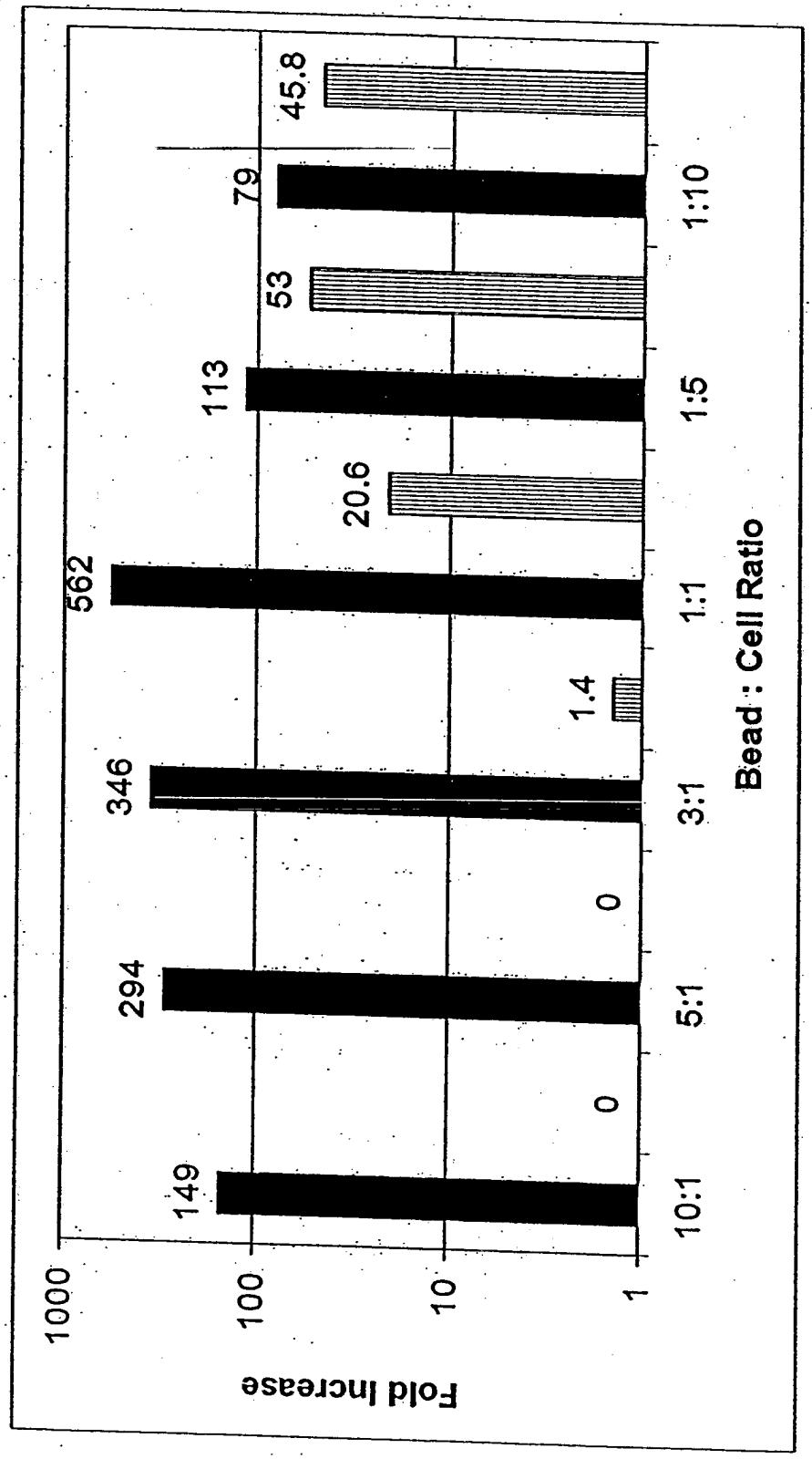

Fold Increase
1000
100
10
1
Bead : Cell Ratio
10:1
5:1
3:1
1:1
1:5
1:10
149
294
346
562
113
79
45.8
53
20.6
1.4
0
0

Fig. 7

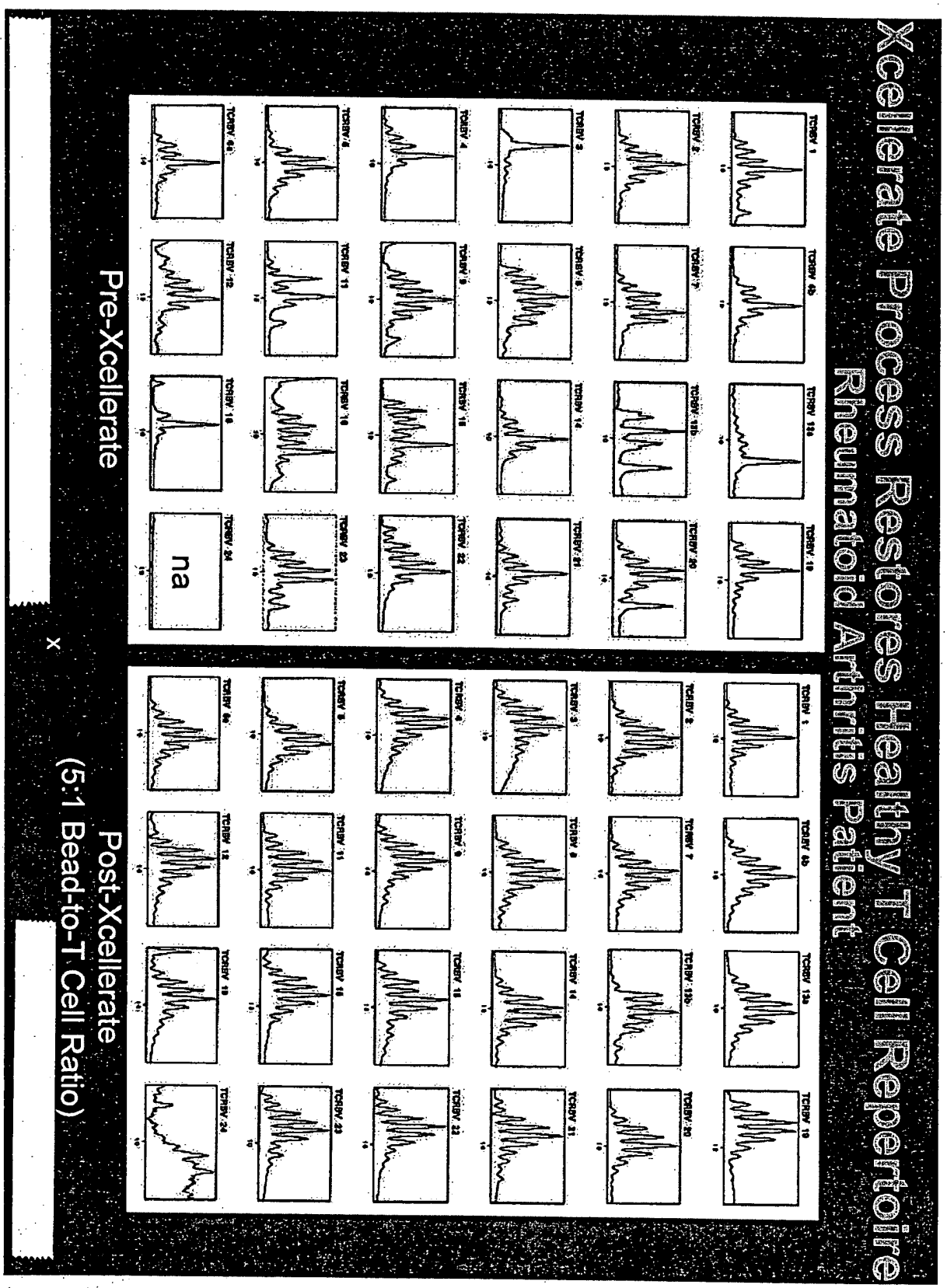

Xcellerate Process Restores Healthy T Cell Repertoire
Rheumatoid Arthritis Patient
Pre-Xcellerate
x
Post-Xcellerate
(5:1 Bead-to-T Cell Ratio)
na

Fig. 8

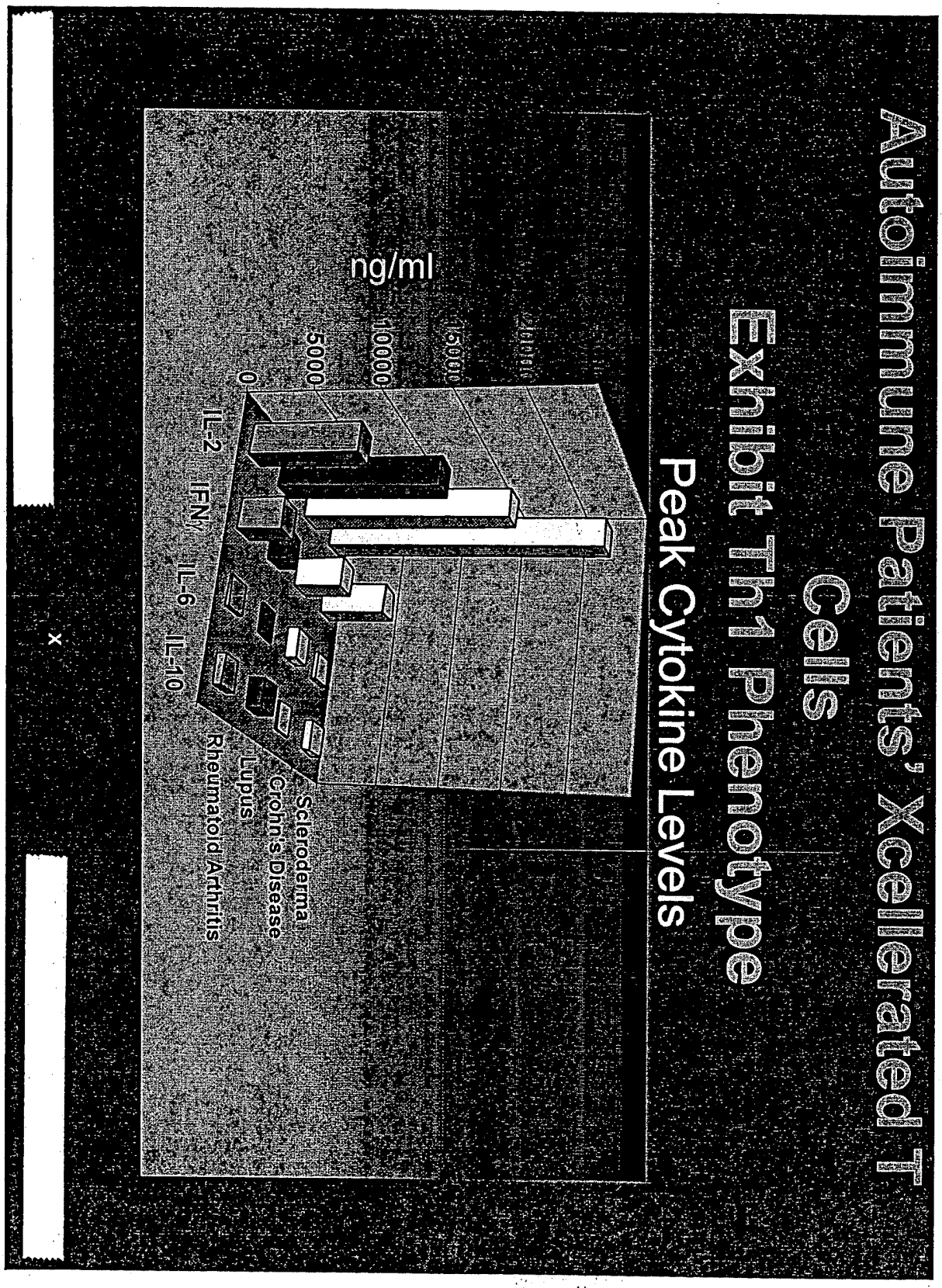

Autoimmune Patients' Xcelerated T Cells
Exhibit Th1 Phenotype
Peak Cytokine Levels
ng/ml
20000
15000
10000
5000
0
IL-2
IFNγ
IL-6
IL-10
Scleroderma
Crohn's Disease
Lupus
Rheumatoid Arthritis

Fig. 9

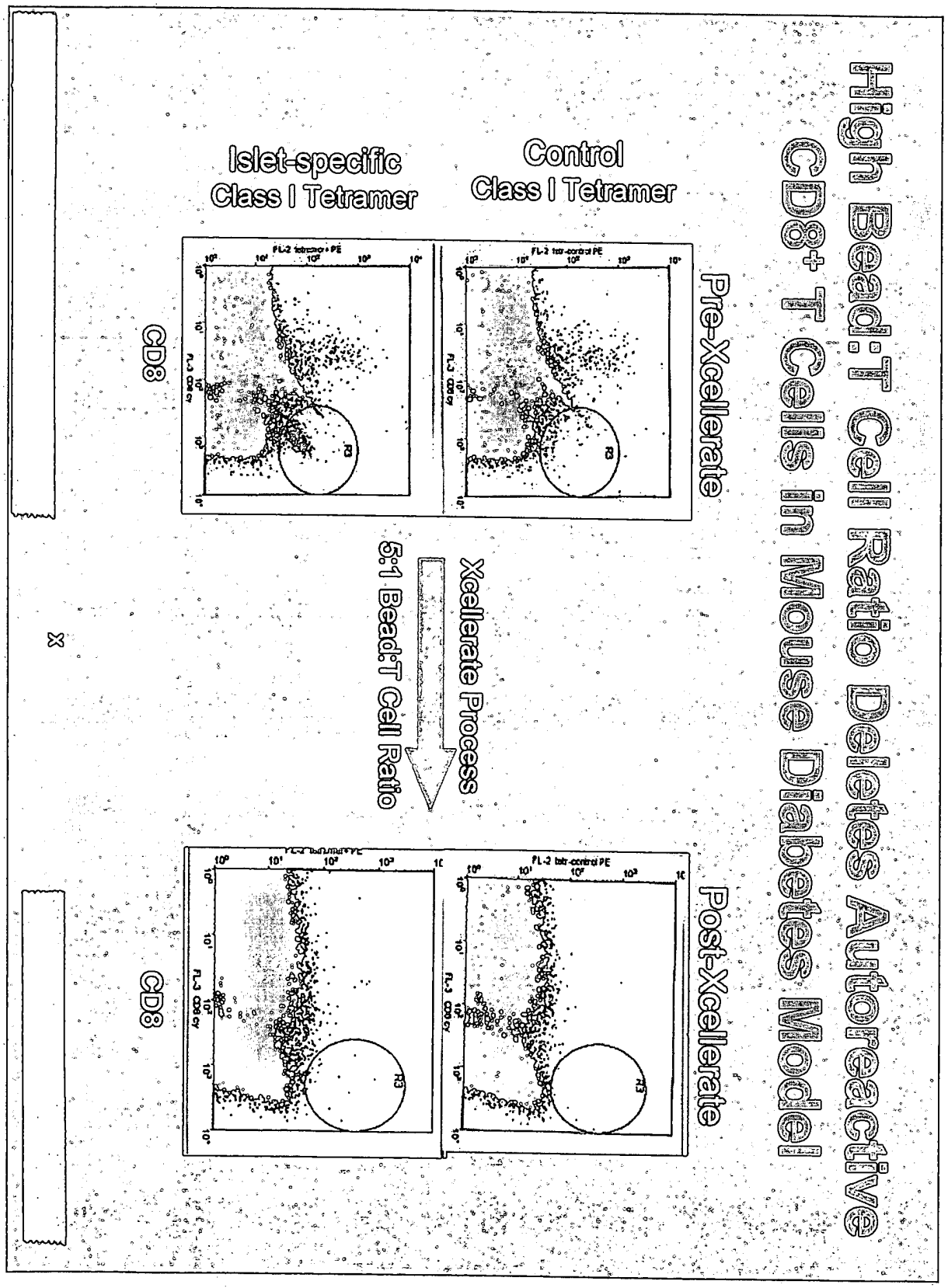

Islet-specific Class I Tetramer
Control Class I Tetramer
CD8
Pre-Xcellerate
5:1 Bead:T Cell Ratio
Xcellerate Process
CD8
Post-Xcellerate
Light Bead:T Cell Ratio Depletes Autoreactive CD8+ T Cells in Mouse Diabetes Model

Fig. 10